**Bacterial Transformation**

**Introduction:**

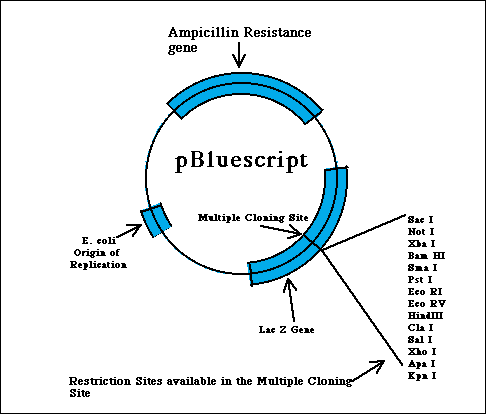
This is a very basic technique that is used on a daily basis in a molecular biological laboratory. The purpose of this technique is to introduce a foreign plasmid into a bacteria and to use that bacteria to amplify the plasmid in order to make large quantities of it. This is based on the natural function of a plasmid: to transfer genetic information vital to the survival of the bacteria.

**The plasmid:**

A plasmid is a small circular piece of DNA (about 2,000 to 10,000 [base pairs](http://faculty.plattsburgh.edu/donald.slish/glossary.html#base%20pairs)) that contains important genetic information for the growth of bacteria. In nature, this information is often a gene that encodes a protein that will make the bacteria resistant to an antibiotic. Plasmids probably came about as a result of bacteria evolving in close proximity to other heterotrophs. Bacteria often grow in the same environment as molds and fungi and compete with them for food (complex organic material). As a result, molds and fungi have evolved to make toxins that kill bacteria (which we now use as antibiotics in medicine) in order to win in the competition for food. Bacteria, in turn, evolved to make proteins that inactivate the toxins. The bacteria share this vital information by passing it among themselves in the form of genes in plasmids.

**Plasmids** were discovered in the late sixties, and it was quickly realized that they could be used to amplify a [gene of interest](http://faculty.plattsburgh.edu/donald.slish/glossary.html#Gene%20of%20Interest). A plasmid containing resistance to an antibiotic (usually ampicillin) is used as a [vector](http://faculty.plattsburgh.edu/donald.slish/glossary.html#vector). The gene of interest is inserted into the vector plasmid and this newly constructed plasmid is then put into E. coli that are sensitive to ampicillin. The bacteria are then spread over a plate that contains ampicillin. The ampicillin provides a [selective pressure](http://faculty.plattsburgh.edu/donald.slish/glossary.html#selective%20pressure) because only bacteria that have acquired the plasmid can grow on the plate. Therefore, as long as you grow the bacteria in ampicillin, it will need the plasmid to survive and it will continually replicate it, along with your gene of interest that has been inserted to the plasmid.

There are many different kinds of plasmids commercially available. All of them contain 1) a selectable marker (i.e., a gene that encodes for antibiotic resistance), 2) an origin of replication (which is used by the DNA making machinery in the bacteria as the starting point to make a copy of the plasmid) and 3) a multiple cloning site. The multiple cloning site has many restriction enzyme sites (to be discussed in a [later lab](http://faculty.plattsburgh.edu/donald.slish/Restmap.html#Restriction%20enzymes)) and is used to insert the DNA of interest. The multiple cloning site is usually in the middle of a reporter gene like [Lac Z](http://faculty.plattsburgh.edu/donald.slish/glossary.html#Lac%20Z). A commonly used plasmid is pBluescript:



**Figure 1**

The main differences among commercially available plasmids are the number of restriction enzyme sites, their order in the multiple cloning site, the type of antibiotic resistance that the plasmid confers, and some other genetic information that makes the plasmid useful for a [specific purpose](http://faculty.plattsburgh.edu/donald.slish/glossary.html#specific%20purpose). Bacteria transformed with pBluescript will survive in ampicillin containing media and will replicate the plasmid, including any gene that is placed in the multiple cloning site.

**Competent Cells:**

Since DNA is a very hydrophilic molecule, it won't normally pass through a bacterial cell's membrane. In order to make bacteria take in the plasmid, they must first be made "competent" to take up DNA. This is done by creating small holes in the bacterial cells by suspending them in a solution with a high concentration of calcium. DNA can then be forced into the cells by incubating the cells and the DNA together on ice, placing them briefly at 42oC (heat shock), and then putting them back on ice. This causes the bacteria to take in the DNA. The cells are then plated out on antibiotic containing media.

For a short animation on E. coli transformation [click here](http://faculty.plattsburgh.edu/donald.slish/Transmovie.html).

**Competency**  
The procedure to prepare competent cells can sometimes be tricky. Bacteria aren't very stable when they have holes put in them, and they die easily. A poorly performed procedure can result in cells that aren't very competent to take up DNA. A well- performed procedure will result in very competent cells. The competency of a stock of competent cells is determined by calculating how many E. coli colonies are produced per microgram (10 -6 grams) of DNA added. An excellent preparation of competent cells will give ~108 colonies per ug. A poor preparation will be about 10 4 / ug or less. Our preps should be in the range of 10 5 to 10 6.

In this experiment you will be making competent cells, transforming them with a plasmid and calculating their competency. There will be a lab report due for this lab.

**Procedure:**

**Important**  
This procedure must be performed under sterile conditions. Use only autoclaved plastic-ware and always work with a flame in front of you. Also, bacteria are very [labile](http://faculty.plattsburgh.edu/donald.slish/glossary.html#labile) in high calcium, so keep the bacteria on ice and away from the flame at all times to keep them viable.

**Competent Cells:**

1. **(This step will be performed for you before you come into lab)**. Pick a single colony from a freshly grown plate of E. coli and disperse it in 100 ml of LB media in a 1 L flask. Incubate the culture at 37oC with vigorous shaking for approximately 3 hours. Cell density is monitored by determining [OD600](http://faculty.plattsburgh.edu/donald.slish/glossary.html#OD600) and should be less than 10 8 cells / ml (log phase of growth - the most healthy bacteria).

2. Transfer 50 ml of this culture to a 50 ml conical tube and centrifuge at 2,000 rpm for 10 min. (Sorvall HS-4 rotor) in room 312.

3. Decant the supernatant into waste beaker (this must be sterilized before being dumped down the drain). Resuspend the pellet in 10 ml of ice cold 0.1 M CaCl2. This is most easily done by resuspending in 1 ml, using the P1000 pipette and then adding 9.0 ml. Cut about 0.5 cm from the end of the blue tip before pulling E. coli through it, since the cells are fragile in this high calcium solution and may lyse if sheared. After they have been resuspended, centrifuge at 2,000 rpm for 10 min. (Sorvall HS-4 rotor).

4. Decant the supernatant into waste beaker (to be autoclaved later). Resuspend the pellet in 1.0 ml of ice cold 0.1 M CaCl2

**Bacterial Transformation:**

1. Pipette 200ul competent cells into each of 3 ice cold Eppendorf tubes. Label the tubes Control, 1 ng, and 10 ng (1 ng is 10 -3 ug, or 10 -9 milligrams). The unknown plasmid is at a concentration of 1 ng/ul. Add 1 ng of your unknown plasmid to one tube and 10 ng to the other. Place the tubes on ice for 30 min.

2. Put the tubes at 42oC for exactly 90 seconds. Return the cells to ice for 1-2 minutes.

3. Pipette the transformation mixtures onto labeled plates containing ampicillin and spread them around using a sterilized, bent glass rod spreader.

4. Place upside down in the 37oC incubator in room 305 overnight.

5. 16 - 20 hours later, count the number of colonies on the plate with well-isolated colonies. Put parafilm around the edge of a plate and put it in a refrigerator for later use. Check the control plate to see that no colonies grew on it. **Dispose of the plate and the control plate in the biohazard bag.**

**Calculations:**

To calculate the competency of your cells, divide the number of colonies on your plate by the amount of DNA (in ug) you added to the transformation.